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Segment No. 03-07-10

WA-07-1010

CITY OF EVERETT CLASS II INSPECTION

by
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ABSTRACT

A Class II inspection was conducted at the Everett Wastewater Treatment Plant on August 12 and 13, 1986. The aerated/faculative lagoon system occasionally experiences NPDES permit violations from overloading. The plant was operating well during the inspection, but experienced minor violations of biochemical oxygen demand, total suspended solids, and pH, as is common to lagoon systems in summer. The trout bioassay indicated acute effluent toxicity, possibly from ammonia or lead.

INTRODUCTION

A Class II inspection was conducted at the Everett Wastewater Treatment Plant (WTP) on August 12 and 13, 1986. The inspection was requested by David Wright of Ecology's Northwest Regional Office (NWRO) to characterize the current status of the plant prior to facility upgrade. Conducting the inspection were Marc Heffner and Don Reif of Ecology's Water Quality Investigations Section, with assistance from Mike Dawda, NWRO. Objectives were to:

1. Collect samples and measure flows to estimate plant loadings and efficiencies.

2. Evaluate metals concentrations in the influent and effluent.

3. Estimate effluent toxicity by performing a series of bioassays.

4. Perform a laboratory evaluation including sample splits, for accuracy and adherence to established techniques.

A receiving water study was conducted at the same time (Determan, 1987).

LOCATION AND DESCRIPTION

The city of Everett's municipal wastewater treatment plant is located on the south end of Smith Island in Snohomish County (Figures 1 and 2). The treatment process consists of influent pumping, mechanically cleaned bar screens, flow measurement, grit removal and washing, two 15-acre aerated lagoons in series, a 135-acre facultative stabilization pond, a 27-acre polishing pond, and chlorine disinfection in an earthen contact chamber. Grit and screenings are hauled to Cathcart Sanitary Landfill.

The plant treats domestic sewage from the city of Everett and surrounding districts. Pretreated and non-pretreated industrial wastewater is also received. In addition, the facility accepts pretreated leachate from Snohomish County landfills.

The plant occasionally experiences NPDES permit violations, mostly from hydraulic and organic overloading. Plans for plant expansion and

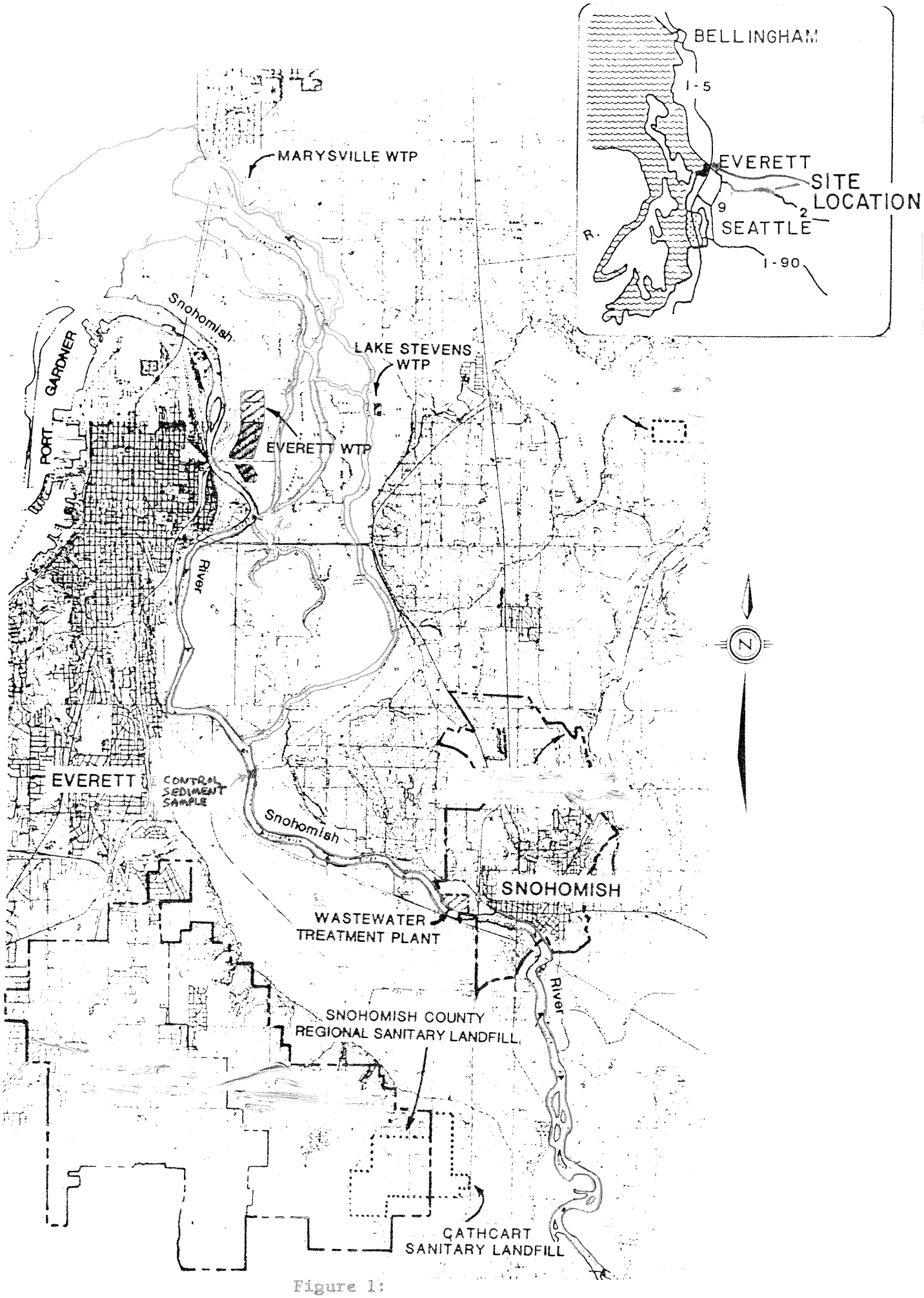
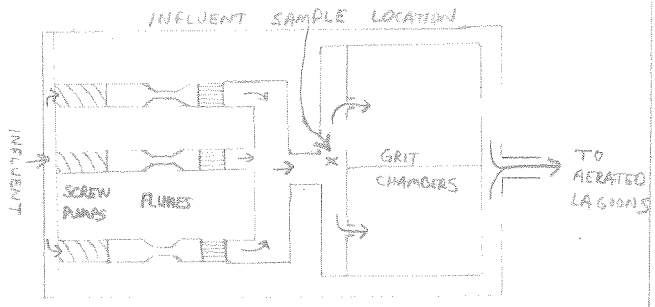


Figure 1:
Site Location and Sediment Control Sample Site



Headworks Enlargement

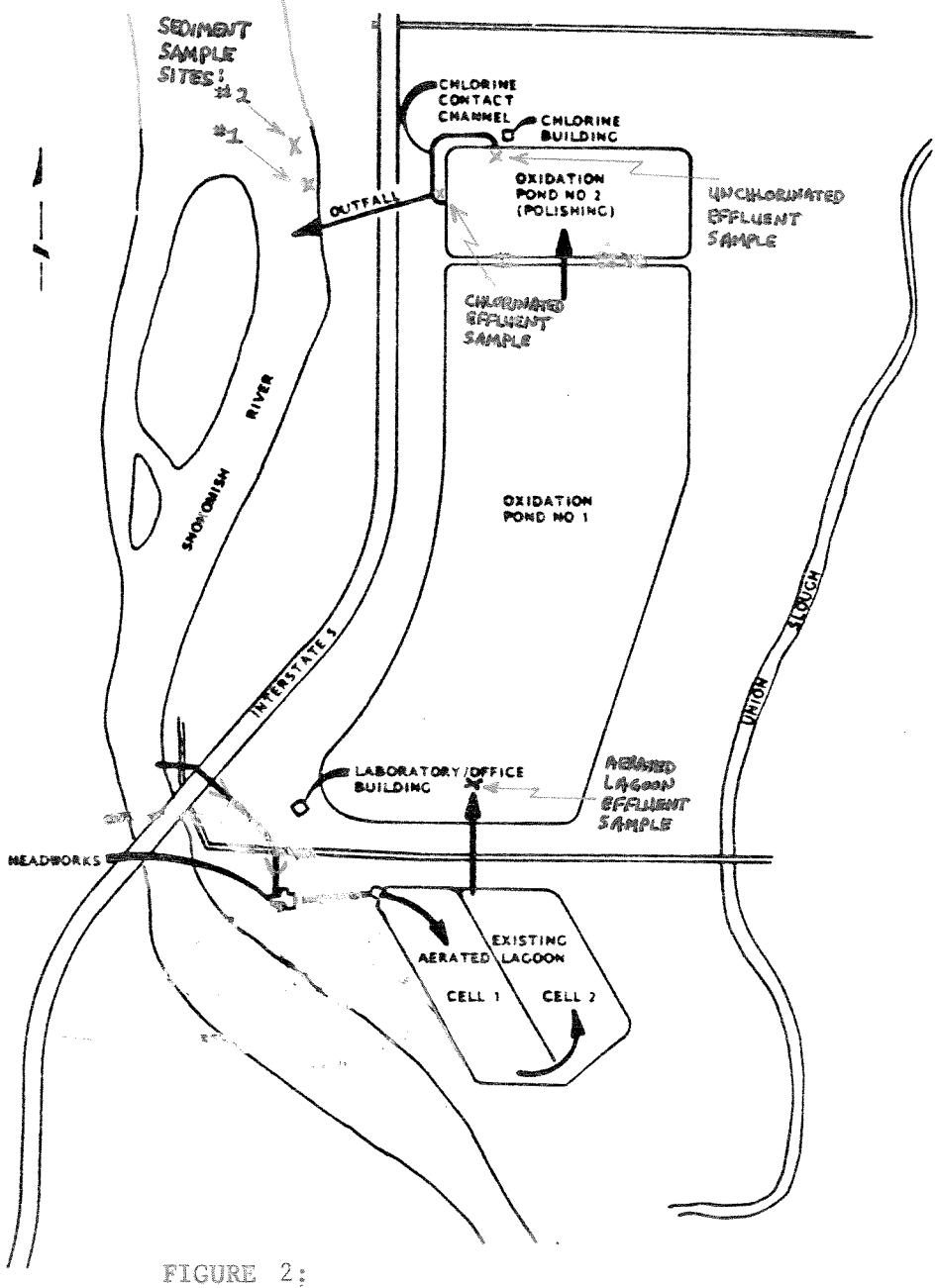


FIGURE 2:
Flow diagram and sampling sites

modification are currently underway. Tentative plans are as follows (Dave Wright, personal communication):

Phase 1: Construct new laboratory and office facilities, a new tide gate, and a facultative lagoon recirculation system. Remove excess sludge from the aerated lagoons.

Phase 2: Expand the existing aerated lagoons, install a scum barrier near the head of the first aerated lagoon, and construct new wetlands. Construct a trickling filter/solids contact (TF/SC) treatment system with clarifier and chlorine contact chamber.

The decision on a new separate outfall for the TF/SC plant has not been made at this time.

METHODS

Plant flow is measured at the headworks with 24-inch Parshall flumes, one per influent channel (Figure 2, inset). Both instantaneous and totalizer readings are recorded in the headworks building control room. Only one channel was in operation during the inspection. The flume's staff gage was compared with the meter's instantaneous flow reading to estimate accuracy.

Plant sampling consisted of composite and grab samples (Table 1). Twenty-four-hour composited samples were collected by both Ecology and Everett from the influent (between bar screens and grit chamber) and effluent, before chlorination. The compositors were set to collect about 200 mL every 30 minutes. Sample collection lines were placed directly beside Everett's sample lines. Ecology grab samples were collected at these same locations and from the facultative lagoon influent pipe (Figure 2).

Cladoceran (Ceriodaphnia dubia) and fathead minnow (Pimephales promelas) chronic bioassays were run at EPA's Environmental Research Laboratory in Duluth, Minnesota (EPA, 1985). Dilutions of 100, 30, 10, 3, and 1 percent effluent, plus control were tested. These two bioassay samples consisted of a three-grab composite of chlorinated effluent. A 96-hour juvenile rainbow trout bioassay (65 percent effluent) was run at Ecology's Manchester Laboratory, in accordance with the department's procedure for "Static Acute Fish Toxicity Test." The trout bioassay grab sample (unchlorinated effluent) was collected August 25, 1986, by Mike Dawda.

Snohomish River sediments were collected with a petite ponar grab sampler. Two sampling sites were located 250 and 500 yards below the outfall, and approximately 20 yards off the east shoreline (Figure 2). Sediment was not collected closer to the outfall because of the extensive rip-rap in the area. A control sample was collected approximately two miles upstream from the plant. Tides are known to carry effluent upstream as far as a mile, but probably no farther (Jones & Stokes Associates, 1986). Sediment samples were strained through a 2mm mesh sieve on-site. Sediment bioassays were then conducted at

Table 1. Ecology sampling schedule, Everett Class II Inspection, August 12-13, 1986.

Sample Type / Station	Date	Time	Field Analysis				Laboratory Analysis													Bioassays				
			Temperature	pH	Conductivity	Cl_2 Residual	Fecal Coliform	Fecal Strep.	pH	Turbidity	Conductivity	Alkalinity	Total Hardness	Sulfate	Solids (4)	NH_3-N	NO_3-N + NO_2-N	T. Phos.-P	Metals	BOD_5	COD	Ceriodaphnia	Fathead Minnow	Rainbow Trout	Hyalella & D. Magna
Composite																									
Influent	8/12-13	1200-1200	X	X	X				X	X	X	X	X	X	X	X	X	X	X	X	X				
Effluent	8/12-13	1200-1200	X	X	X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Sediment	8/14	a.m.																							X
Ecology Grabs																									
Influent	8/12	a.m.	X	X	X																X				
	8/12	p.m.	X	X	X				X	X	X	X	X	X	X	X	X	X			X				
	8/13	a.m.	X	X	X				X	X	X	X	X	X	X	X	X	X			X				
Aerated Lagoon Effluent	8/12	a.m.	X	X	X																X				
	8/12	p.m.	X	X	X				X	X	X	X	X	X	X	X	X	X			X				
	8/13	a.m.	X	X	X				X	X	X	X	X	X	X	X	X	X			X				
Effluent	8/12	a.m.	X	X	X	X	X	X													X				
	8/12	p.m.	X	X	X		X		X	X	X	X	X	X	X	X	X	X			X				
	8/13	a.m.	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X			X				

EPA's Corvallis, Oregon, Environmental Research Laboratory, with both Daphnia magna (48 hour) and Hyalella azteca (96 hour). Analysis consisted of 3 replicate beakers per sample, with 10 Daphnia and 20 Hyalella per beaker. Each 1-liter beaker contained 200 mL of sediment, plus 700 mL of well water (Nebeker, et al., 1984).

RESULTS AND DISCUSSION

Flow Measurement

Flow data are presented in Table 2. Flow was estimated at 10.55 MGD based on Everett's records from 8:00 a.m., August 12, until 8:00 a.m., August 13. This flow was used for all calculations. Two instantaneous measurements indicated a slight discrepancy between the flow calculated from the flume's staff gage and the flow indicated on the control panel.

Table 2. Flow data - Everett Class II Inspection, August 12-13, 1986.

		Ecology Instantaneous Measurement		Plant Meter	
Date	Time	Height (ft)	Flow (MGD)*	Instantaneous	Totalizer
8/12	1325	1.67	11.4	12.0	5289.96
8/13	0913	---	---	---	5298.10
8/13	1305	1.79	12.7	13.8	5300.30

Total flow (plant meter): 0800 - 0800 hours = 10.55 MGD
*From Leupold & Stevens, 1978.

NPDES Permit Compliance

At the time of the inspection, the Everett plant was operating under NPDES discharge permit WA-002449-0(M). This permit expired June 19, 1982, but remained in effect until the new permit (WA-002449-0) was issued on November 18, 1986. The new permit has two phases. Interim limitations are in effect until the lagoon upgrade is completed. Final limitations and monitoring requirements will then become effective until November 18, 1991.

Analytical results are listed in Table 3. Table 4 compares inspection data to the old permit, in effect at the time of the inspection. Most parameters were well within permit limitations. Officially, several values exceeded weekly average limitations: percent removal of biochemical oxygen demand (BOD) and suspended solids, and one of three pH samples. Because these violations are most likely related to typical seasonal algal blooms within the treatment lagoons, they are not considered serious.

Table 3. Ecology sample results - Everett Class II Inspection, August 12-13, 1986.

Sample	Sampler	Date	Time	Field Analysis: Temperature (°)	pH (S.U.)	Conductivity (umhos/cm)	Ecology Laboratory Analysis: pH (S.U.)	Turbidity (NTU)	Conductivity (umhos/cm)	Alk. (mg/L as $CaCO_3$)	Total Hardness (mg/L as $CaCO_3$)	Sulfate (mg/L as SO_4)	Solids (mg/L): Total	Total Non-volatile	Total Suspended	Total Non-volatile Suspended	Nutrients (mg/L): NH_3-N	NO_3-N + NO_2-N	Total Phosphorus	BOD_5 (mg/L unchlor)	COD (mg/L)	Fecal Coliform (#/100 mL)	Fecal Strep. (#/100 mL)
Composite Samples																							
Influent	Ecology	8/12-13	1200-1200	6.0	7.8	510	7.2	30	489	130	57	27	490	190	190	27	16	0.07	2.8	200	410		
	Everett	8/12-13	0800-0800				7.3	28	461	130	64	27	440	200	250	34	16	0.07	2.9	180	380		
Effluent	Ecology	8/12-13	1200-1200	--	--	--	9.0	9	430	120	67	22	330	170	42	1	11	0.24	2.7	12	100		
	Everett	8/12-13	0800-0800				8.6	8	439	130	66	24	300	180	45	4	10	0.37	2.7	<100	100		
Ecology Grab Samples																							
Influent		8/12	1140	19.7	6.8	420	--	--	--	--	--		--	--	--	--	--	--	--		440		
		8/12	1605	19.9	6.9	460	6.9	21	453	140	49		490	220	200	54	17	0.04	2.9		380		
		8/13	0905	18.9	6.9	405	7.2	22	454	130	68		330	130	110	14	17	0.05	2.4		240		
Aerated		8/12		--	--	--	--	--	--	--	--		--	--	--	--	--	--	--		120		
Lagoon		8/12	1630	20.6	7.2	550	7.1	5	523	160	68		290	170	41	6	20	0.04	2.9		140		
Effluent		8/13	0920	20.6	7.2	525	7.3	9	534	170	70		300	200	33	13	20	<0.01	2.7		110		
Effluent		8/12	1227	22.4	9.0	375	--	--	--	--	--		--	--	--	--	--	--	--		100	23	29
		8/12	1650	25.1	9.3	405	9.5	7	415	120	77		300	180	50	5	11	0.25	2.8		100	28	
		8/13	0930	22.1	8.8	410	9.2	7	420	130	74		300	200	37	3	10	0.26	2.7		93	5	24

Table 4. Comparison of inspection data to NPDES permit limits - Everett Class II Inspection, August 12-13, 1986.

Parameter	Effluent Limitations			Inspection Data*		
	Weekly Average	Monthly Average	Daily Maximum	Ecology Composite	Everett Composite	Ecology Grab
BOD_5 - mg/L	45	30	--	12	28	--
- lbs/day**	11,600	7,750	--	1,056	2,464	--
- % removal	85	--	--	94	84***	--
TSS - mg/L	45	30	--	42	46***	--
- lbs/day**	11,600	7,750	--	3,695	4,047	--
- % removal	85	--	--	78***	71***	--
Fecal Coliforms (#/100 mL)	400	200	--	--	--	23;28;5
Chlorine Residual (mg/L)	--	0.5	--	--	--	0.2;0.2
Chromium, Total (mg/L)	--	--	0.1	<0.001	0.0036	--
Copper (mg/L)	--	--	0.1	0.014	0.009	--
Zinc (mg/L)	--	--	0.1	0.039	0.041	--
pH	$6.0 \leq pH \leq 9.0$			--	--	9.0; 9.3***; 8.8

*Ecology composite data are based on Ecology laboratory results; Everett composite data are based on Everett laboratory results; grab samples listed are Ecology laboratory results.

**Based on flow of 10.55 MGD.

***Exceeds weekly average effluent limitations.

Chlorine residual was measured at the chlorine contact chamber discharge. The residual was difficult to read colorimetrically due to the green tint of the effluent. Estimated total residual was 0.2 mg/L each for two samples tested.

The three permitted metals (chromium, copper, and zinc) were well within allowable limits. Hexavalent chrome was analyzed by Hach kit in the field: none was detected.

General Parameters

The Everett plant seemed to be functioning as a typical lagoon treatment system, including seasonal algal blooms. The vast majority of pollutant reduction seemed to occur in the aerated lagoons. A comparison of aerated lagoon effluent values to Everett's final effluent shows mostly unremarkable changes occurred in the facultative lagoons. Most parameters remained very similar: some decreased modestly, while others, such as pH and TSS, increased. COD decreased slightly and thus probably BOD, even though suspended solids were higher.

Ammonia levels were reduced nearly 50 percent during facultative lagoon treatment: nitrate increased only slightly. Therefore, the ammonia loss may have been due to algal uptake, ammonia volatilization, or other processes, rather than biological nitrification. Total phosphorus remained essentially unchanged.

Metals

Effluent metals did not exceed the limits of the permit in effect during the inspection. The new permit adds limits for lead, and requires monitoring of four additional heavy metals--nickel, mercury, silver, and cadmium.

Because these metals were to be included in the new permit, they were analyzed during the inspection. Results are listed in Table 5. Effluent lead exceeded by several times the proposed concentration and daily loading limits.

BIOMONITORING

Effluent

Results of the effluent bioassays are listed in Tables 6, 7, and 8. The Ceriodaphnia test was not valid because of unacceptably low control survival--70 percent (Table 6). A minimum of 80 percent survival is required for this test.

Table 5. Influent and effluent metals: Ecology analysis of Ecology samples - Everett, August 1986.

Metal	Influent ug/L	Effluent ug/L	Effluent lbs/day**	New Permit Limit* ug/L	New Permit Limit* lbs/day
Chromium, Total	10	<1	<0.09	100	8.8
Copper	108	14	1.2	30	2.65
Zinc	161	39	3.4	420	37.1
Lead	24	20***	1.8***	1.4	0.12
Cadmium+	1.9	0.9	0.08	--	--
Mercury+	0.25	<0.05	<0.004	--	--
Silver+	9.2	1.1	0.095	--	--

*Limits, as listed, are interim requirements on new permit; were not in effect during this survey.
**Based on flow of 10.55 MGD.
***Exceeds new permit limit. Lead was not a permitted parameter during this inspection.
+For monitoring only; no permit limits are established.

Table 6. Ceriodaphnia dubia results: Everett Class II Inspection, August 12-13, 1986.

Percent Effluent	Mean Number of Young per Female	Seven-Day Percent Survival
Control	15.2	70
1	17.0	100
3	16.5	100
10	14.8	100
30	20.1	80
100	11.5	100

Table 7. Fathead minnow results: Everett Class II Inspection, August 12-13, 1986.

Percent Effluent	Weights, mg	Percent Survival
Control	0.718	100
1	0.599	100
3	0.842	45
10	0.738	90
30	0.796	95
100	0.548	85

Table 8. Rainbow trout bioassay results: Everett Class II, August 12-13, 1986.

Percent Effluent	Percent Survival
Control	100
65	20

The fathead minnow bioassay resulted in no significant mortality or growth reduction due to Everett effluent (Table 7). Although survival was low at the 3 percent dilution, effluent toxicity was probably not the cause (Amato, 1987). Slightly decreased survival and growth at 100 percent effluent was not statistically significant as compared to the control.

The rainbow trout bioassay resulted in only 20 percent survival, indicating significant acute toxicity (Table 8). Two possible causes are lead and ammonia. Lead concentrations were 2 to 6 times greater than EPA's water quality criteria for four-day average concentrations: 3.9 ug/L (Everett analysis) or 11.7 ug/L (Ecology analysis), versus the 1.9 ug/L criterion (EPA, 1986).

A likely cause for the trout mortality was ammonia toxicity. The allowable ammonia concentration decreases drastically at higher pH values. Lagoon systems, such as the Everett WTP, can have elevated pHs due to algal activity. The trout bioassay sample had a pH of 9. The total ammonia criteria, at pH 9, are 0.68 and 0.13 mg/L for one-hour and four-day average concentrations, respectively. Total ammonia in the test solution was thus about 10 and 55 times greater than these respective criteria.

All trout mortalities occurred within the first 24 hours. After 24 hours, the test solution pH had dropped from 9 to 7.2. The ammonia criterion at this pH is much higher than the concentration in the test solutions.

Sediments

Sediment bioassay results are listed in Table 9. No sediment toxicity from Everett's effluent was apparent. Hyalella azteca showed no significant toxicity in either the control or downstream samples. Daphnia magna showed significant mortality in the control and downstream #2 samples, plus some mortality in sample #1. Since Hyalella showed neither mortality nor stress, it appears that Daphnia's low survival may be attributed to factors unrelated to Everett's discharge.

Table 9. Sediment bioassay results with Daphnia magna and Hyalella azteca: Everett Class II, August 12-13, 1986.

Parameter	Percent Survival	
	Daphnia	Hyalella
Control	0	95
Downstream #1	77	95
Downstream #2	7	97

Several factors could have contributed to the problems observed with the Daphnia bioassay. In the control, Daphnia's problem may have been related to very soft water: hardness was only 33 mg/L, far below Daphnia's optimal range of 200 to 400 mg/L. Daphnia's downstream survival problems may have been due to salinity. Hyalella is a more salt-tolerant organism than Daphnia (Nebeker, personal communication).

Laboratory Review

The Everett lab appeared well organized and efficiently operated, although cramped for space. Analytical techniques seemed to follow accepted protocols very well. Two recommendations are made:

1. All composite sampler lines should be cleaned regularly with a chlorine bleach and water solution. A suggested routine is to clean the influent lines weekly and effluent lines monthly.

2. Distilled water for BODs should be aged for one week before use. This may reduce the occasional problem of high dilution blank oxygen depletion.

The sample splits between Ecology's and Everett's laboratories showed generally very good agreement, especially for the effluent samples (Table 10). Both labs got higher BOD and TSS values from Ecology's influent sample. Implementation of recommendation #1 may correct this.

SUMMARY AND RECOMMENDATIONS

The city of Everett's wastewater treatment plant was operating well at the time of the inspection. Percent BOD and TSS, and effluent pH exceeded weekly average permit limits. Algal productivity appeared to be a contributing factor. These violations, therefore, are not considered serious.

Effluent bioassays indicated significant acute toxicity in the 96-hour trout test. Ammonia and lead were identified as possible causes. No significant toxicity was observed from the fathead minnow bioassay. The Ceriodaphnia test was not valid due to excessive control mortality.

No toxicity was apparent in Snohomish River sediments as a result of Everett's discharge. The results were, however, inconclusive, and further testing is recommended. Concurrent chemical analysis of the sediment is strongly recommended to aid in interpretation.

The plant's laboratory appeared to be well run. Analytical technique in general was good, and sample splits between the Ecology and Everett laboratories compared quite well. Two recommendations were made in the discussion section.

Table 10. Summary of split sample conventionals and metals data from Everett Class II Inspection: August 12-13, 1986. All metals expressed in ug/L.

	Influent				Unchlorinated Effluent				Chlor. Effluent	
	Ecology Analysis		Everett Analysis		Ecology Analysis		Everett Analysis		Ecology Analysis	Everett Analysis
Parameter	Ecology Comp.	Everett Comp.	Ecology Comp.	Everett Comp.	Ecology Comp.	Everett Comp.	Ecology Comp.	Everett Comp.	Ecology Grab	Everett Grab
BOD_5, mg/L	200	165	180	171	12	<100	16	28		
TSS, mg/L	190	152	250	161	42	42	45	46		
Chromium, Total	10	10	11.4	6.8	<1	<1	3.3	3.6		
Copper	108	153	65	65	14	10	10	9		
Zinc	161	291	174	196	39	15	41	41		
Lead	24	67	21	21	20	16	6	6		
Cadmium	1.9	2.7	0.4	0.7	0.9	0.9	0.1	0.2		
Nickel	11	13	11	12	20	<1	2	2		
Fecal Coliform (#/100 mL)									5	6
Fecal Streptococci (#/100 mL)									24	18

REFERENCES

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APPENDIX

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
CORVALLIS ENVIRONMENTAL RESEARCH LABORATORY

WESTERN FISH TOXICOLOGY STATION
1350 S.E. GOODNIGHT AVE.
CORVALLIS, OREGON 97330

Don Reif
Dept. of Ecology
7272 Clearwater Lane
LU-11
Olympia WA 98504-6811

Aug. 25, 1986

Dear Don,

Enclosed are the results of our sediment bioassays:

Daphnia magna and the amphipod Hyalella azteca were the test organisms. We used Daphnia knowing that water might be too soft for them to survive in. We used our well water, with the following chemical characteristics: conductivity - 112 µs
pH - 6.2
hardness - 33 mg/L as $CaCO_3$
alkalinity - 33 mg/L as $CaCO_3$

The animals were tested in 1000 ml beakers, which contained 200 ml sediment and 700 ml well water. The animals were tested together in the same beaker. We used 3 replicate beakers per sample with 10 Daphnia and 20 Hyalella in each beaker. Daphnia were 4 days old, Hyalella were adults.
Temperature was 20 ± 1°C. Photoperiod - 15 light : 9 dark

Hyalella test started Aug. 18, 1986, ended Aug. 22.
Daphnia tested from Aug 19 to 21 in same beakers with Hyalella.
Daphnia and most Hyalella were removed from the jars with eye dropper (5 mm ID) or forceps. Remaining mud + Hyalella was poured thru a 1 mm mesh screen to recover Hyalella.
These tests were run concurrently with those for Dave Tetpenning from the Coeur d'Alene so conditions were the same.

Site	Beaker	No. Daphnia alive after 48-hr (n=10)		No. Hyalella alive after 96-hr (n=20)		pH after 2 days exposure	Hardness after 4 days exposure
Control (upstream) Everett Class II 8/19, AM, DCR	A	0		20		7.28	33 mg/L
	B	0		20	95%	7.32	-
	C	0		17		7.29	-
Downstream # 1	A	6		19		7.68	267 mg/L
	B	8	77%	20	95%	7.70	278 "
	C	9		18		7.69	-
Downstream # 2	A	0		20		7.70	-
	B	0	7%	18	97%	7.70	-
	C	2		20		7.67	416 mg/L

No predators were found in control sediment. The water is too soft for long term survival and reproduction but we're surprised they all died so rapidly. Is there a source of pollution above the control site. The Hyalella did fine so it may be the soft water??

Downstream # 1 seems to be OK, but obviously enriched because of the higher pH and hardness. No predators were found.

Downstream # 2 gives mixed signals again. Something is toxic to the Daphnia, as they can handle 400 hardness, in fact they prefer 400 to 200. The Hyalella seem to be OK. No predators were found.

The dead were scavenged by the Hyalella, indicating that their appetites were OK, telling us they were not sickened by anything from the sediment.

I'll be back in the lab. Sept. 8 - call me if you like.

Alan V. Nebeker

Alan V. Nebeker

Research Aquatic Biologist

cc: Peterson
Gakstatter

ANDREA BEATTY RINIKER
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M_E_M_O_R_A_N_D_U_M

September 3, 1986

To: Don Reif

From: Barbara Smith, Manchester Laboratory

Subject: Everett STP, Everett
96-Hour Bioassay Information.

Sample_Identification

Laboratory Reference Number: 357230
Date Sample Collected: August 25, 1986
Date Sample Received: August 25, 1986
Sample Submitted by: Don Reif
Sample Description: Clear, green effluent.
Sample collected was not chlorinated.

Test_Procedure

The sample was tested for toxic properties in accordance with the Department of Ecology procedure for "Static Acute Fish Toxicity Test."

Test_Results

The test data are tabulated in detail on the following page(s).

Test_Details

The sample was tested at 65% effluent.

The test organisms were rainbow trout (*Salmo_gairdneri*). The organism length ranged from 24 to 36 mm, giving a short-to-long ratio of 1.5. The mean length was 30.8 mm. The average weight was 0.26 grams.

Ten trout were added to 10 liters of sample/water mixture in each aquarium. This gave a flesh-to-mixture ratio of 0.26 gram/liter.

The test was started on August 25, 1986 at 1400 hours and completed on August 29, 1986 at 1400 hours.

Conclusions

65% effluent- 24/30 fish died= 80 % mortality.
Control- 0/30 fish died= 0 % mortality.

cc: Merley McCall

State of Washington Department of Ecology

DATA SHEET FOR STATIC BASIC ACUTE FISH TOXICITY TEST*

Laboratory: Manchester
Analyst: Barbara Smith

Industry/Toxicant: Everett STP
Address: Everett, WA.
Collector: Mike Dawda for Don Reif
Date Sample Collected: August 25, 1986

Beginning: Date Aug. 25, 1986 Time 2:00 pm.
Ending: Date Aug. 29, 1986 Time 2:00 pm.
Test Organism: Rainbow Trout
Required Test Temperature Range: 12° C ± 1°

Laboratory Reference Number	Test Container No.	Conc. (mg/l)	Number of Cumulative Deaths					Dissolved Oxygen (mg/l)					pH 25 C					Temperature (C)					Total Hardness (mg/l as $CaCO_3$)		Total Alkalinity (mg/l as $CaCO_3$)		Conductivity uMHOS/cm	
			0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	96	0	96	0	96
357230	2	65%	0	8	8	8	8	9.6	8.4	-	-	9.4	9.05	7.22	-	-	8.33	12.5	12.1	-	12.4	12.7	68	70	130	128	364	371
	11	ff.	↓	9	9	9	9	9.6	-	8.9	-	9.4	9.01	-	7.23	-	8.58	↓	↓	-	↓	↓	-	62	109	104	356	368
	12	↓	↓	7	7	7	7	9.6	-	-	9.0	9.6	9.01	-	-	8.71	8.67	↓	↓	-	↓	↓	-	70	109	104	362	373
Control	1	De-Cl	0	0	0	0	0	9.2	8.5	-	-	9.3	8.31	7.14	-	-	8.38	12.5	12.1	-	12.4	12.7	88	62	88	88	239	246
	5	Tap	↓	↓	↓	↓	0	9.1	-	8.2	-	8.7	8.40	-	7.11	-	7.81	↓	↓	-	↓	↓	68	70	84	87	241	247
	14	Water	↓	↓	↓	↓	0	9.1	-	-	8.0	9.1	8.31	-	-	7.90	7.89	↓	↓	-	↓	↓	-	66	88	91	243	248

Sample Description: Clear green effluent - Sample collected was not Chlorinated
Average Weight: 0.269 Mean Length: 30.8 mm Longest: 36 mm Shortest: 24 mm Ratio (long/short): 1.5
Number of organisms per chamber: 10 Ratio of flesh to water: 0.26 g/l. Comments:

* Method on file with the Department of Ecology:
GENERAL PROCEDURE FOR STATIC BASIC ACUTE FISH TOXICITY TEST

ECY 030-1-40

DATA VERIFIED BY: Pam Carey DATE: 9/18/86

_____ EHW > 10/30
_____ DW > 11/30

A Report on the Chronic Toxicity of the Everett, Washington Sewage Treatment Plant Effluent to *Ceriodaphnia dubia* and Larval Fathead Minnows (*Pimephales promelas*)

by

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Falls Church, VA

ERL-DUL-2020

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Introduction

Toxicity tests were conducted on one sample of Everett, Washington Sewage Treatment Plant (STP) effluent. The purpose of the tests was to assess the effluent's toxicity to aquatic life. The tests were utilized to evaluate chronic reproductive effects on Ceriodaphnia dubia and sub-chronic growth effects to larval fathead minnows (Pimephales promelas).

Methods

The effluent sample was collected August 12-13, 1986, as a 24 hour composite and sent to ERL-Duluth by overnight express mail. Samples were shipped in sealed, one gallon polyethylene containers packed in wet ice. The tests were initiated August 18.

The Ceriodaphnia test was set up following the methods of the U.S. EPA manual "Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms," 1985. Thirty milliliter plastic cups were used as the test chambers. Test solutions were warmed in a water bath to 25°C. Fifteen milliliters of test concentration were placed in each of ten plastic cups. The cups were randomly distributed within a styrofoam holding board. Dissolved oxygen, pH and conductivity measurements were taken before animals were transferred to the test water. Table 1 contains water chemistry means and ranges.

One daphnid, $\leq$ six hours old, from ERL-Duluth culture stock was placed in each cup and fed 100 microliters of a yeast, trout food, and Cerophyl mixture daily (13.3 mg/l final concentration of suspended solids).

The test water was renewed on days three and five; and young, if present, were counted and discarded. Final dissolved oxygen measurements were taken on all samples after the animals were transferred into new test water.

Temperature throughout the tests was maintained at 25°C ± 1°C. Effluent concentrations tested were 100%, 30%, 10%, 3%, 1%, and a control. Lake Superior water was used as the diluent.

Ceriodaphnia statistical analysis was performed using the procedure of Hamilton (1984) as modified by Rogers of ERL-Duluth. In this procedure, the young production data were analyzed to obtain the mean number of young per female per treatment. Daily means were calculated, and these means were summed to derive the 7-day mean young value. By this method, any young produced from females that die during the test are included in the mean daily estimate. Using this procedure, mortalities of the original females affect the estimate minimally, but the mortality of the adult is used along with the young production to determine overall toxicity effects. Confidence intervals are calculated for the mean reproductivity using a standard error estimate calculated by the bootstrap procedure. The bootstrap procedure subsamples the original data set (1000 times) by computer to obtain an estimate of standard error.

A Dunnett's two-tailed t-test is performed with the effluent test data to compare each treatment to the control for significant differences.

A fathead minnow larval growth test was conducted on the effluent sample. Effluent concentrations tested were identical to that of the Ceriodaphnia test. Lake Superior water was used as the diluent. Two hundred and fifty milliliters of test solution was placed in each duplicate chamber. The chambers were assigned random positions. Test solutions were renewed daily. Dissolved oxygen, conductivity, and pH measurements were taken prior to and just after solution renewals. See Table 1 for means and ranges. Temperature throughout the test was maintained at 25° ± 1°C.

Ten fathead minnow larvae, ≤ 24 hours old, from the ERL-D's culture were randomly assigned to each chamber. The chambers measured 18 cm x 6.5 cm

x 9 cm deep with a stainless steel screen glued at a point 2.5 cm from one end of the chambers. This left a narrow sump 2.5 cm x 6.5 cm x 9 cm deep which facilitated solution renewal with minimum disturbance of the fish. Solution depth was 2.5 cm.

Each day 0.1 mls of newly hatched brine shrimp (Reference Artemia) was fed three times and larval survival was counted. The chambers were siphoned daily, prior to solution renewal, to remove dead brine shrimp.

At the end of the test, the fish were killed and preserved in 70% ethyl alcohol. Prior to weighing, the fish were rinsed with distilled water, oven dried at 100°C for 18 hours, and finally weighed on an analytical balance.

The fathead minnow weight analysis assumes the variability in the mean treatment response is proportional to the number of fish per treatment. MINITAB (copyright Pennsylvania State University 1982) was used to estimate a t-value for comparing the mean treatment and control data using weighted regressions with weights equal to the number of replicates per treatment. The t-value is then compared to the critical t-value for the standard two-tailed Dunnett's Test (Steel and Torrie 1960). The survival data are arcsine-transformed prior to the analyses to stabilize variances for percent values.

An Acceptable Effluent Concentration (AEC) is then determined by calculating the geometric mean of the Lowest Observable Effect Concentration (LOEC) and the No Observable Effect Concentration (NOEC).

Results

Table 2 contains the data obtained from the Ceriodaphnia test. The test is not valid due to unacceptable control survival.

The fathead minnow values are contained in Table 3. Low survival in replicate B at the 3% effluent concentration does not seem to be linked to effluent toxicity. None of the values are significantly lower than the control values.

The AEC based on the fathead minnow values is greater than 100%.

Table 1
Routine Chemistry Means and Ranges for
Everett, WA STP Effluent Tests

Percent Effluent	Initials Ceriodaphnia and Fathead Minnow pH Range	DO x	DO Range	Conduct. x	Final Ceriodaphnia pH Range	DO x	DO Range	Final Fathead Minnow pH Range	DO x	DO Range
Control	7.7	8.5	8.3-8.9	94	7.6-7.7	8.0	-	7.2-7.4	6.8	6.2-7.6
1	7.7	8.5	8.3-8.7	98	7.6-7.7	8.0	7.9-8.0	7.2-7.5	6.7	6.3-7.6
30	7.8-7.9	8.6	8.4-8.9	190	7.8	7.9	-	7.2-8.0	7.3	6.0-8.1
100	8.0-8.3	8.7	8.3-8.9	393	7.8-7.9	7.8	-	7.4-7.8	7.4	6.1-8.5

Table 2
Mean Number of Young per Female and Percent Survival for
Ceriodaphnia dubia After Seven Days Exposure to
Everett, WA STP Effluent

Percent Effluent	Mean Number of Young per Female	95 Percent Confidence Interval	Seven Day Percent Survival
Control	15.2	9.3-21.1	70
1	17.0	11.9-22.1	100
3	16.5	13.2-19.8	100
10	14.8	10.6-19.0	100
30	20.1	13.6-26.6	80
100	11.5	7.9-15.1	100

Table 3
Individual Mean Dry Weights (mg) and Percent
Survival for Fathead Minnows after Seven Days Exposure
to Everett, WA STP Effluent

Weights

	Percent Effluent					
Replicate	Control	1	3	10	30	100
A	0.736	0.661	0.860	0.703	0.826	0.499
B	0.699	0.536	0.700	0.772	0.762	0.603
Weighted Mean	0.718	0.599	0.842	0.738	0.796	0.548
Standard Error	0.040	0.040	0.060	0.042	0.041	0.044

Survival

A	100	100	80	90	100	90
B	100	100	10	90	90	80
Mean	100	100	45	90	95	85